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PHAGOCYTOSIS OF MELANIN PARTICLES BY HUMAN EPIDERMAL CELLS *IN VITRO**

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It is now quite generally accepted that only melanocytes, the specialized cells of melanin synthesis, are capable of synthesizing this polymeric pigment. It is also evident that this pigment, after its formation in the melanocytes of the epidermis, normally finds its way into the epidermal cells where it may persist during their outward migration and exfoliation.

The precise mechanism by which the pigment particles (the melanized melanosomes) are transferred *in vivo* from the donor to the receptor cells is not entirely clear. It has been variously proposed that the pigment particles are first liberated into the intercellular space and then phagocytized by the epidermal cells (1), or that the melanocytes actively inject the particles into the epidermal cells (2). More recent electron microscopic studies of epidermal cells show that the resident pigment particles are frequently enclosed by a membrane which has been interpreted as the wall of the dendrite of the melanocyte (3, 4), implying a phagocytosis of a portion of the dendritic process. The transfer of pigment particles by melanocytes to epidermal cells has been directly observed in mixed cell culture by several workers, and in some instances studied by time lapse cinemicrography (5, 7). We report here on the case of human epidermal cells in cultures, to which partially purified melanin particles were added.

EXPERIMENTAL

Adult human skin was scraped free from the dermal components and treated with trypsin to provide a source of free epidermal cells. These were cultured by the method described by Karasek (8). Melanin suspensions were then added to the culture medium from 24–48 hours after seeding.

Mouse melanoma melanoprotein particles were obtained by homogenizing a deeply pigmented mouse melanoma, differentially centrifuging, and repeatedly washing with sterile saline. The diluted melanin suspension was given a five minute exposure to a medium pressure mercury arc (Hanovia-Alpine) at 10 cm for the purpose of eliminating bacterial contaminants. Aliquots of the suspension were dried on microscope slides, and stained with hematoxylin and eosin. Microscopic examination showed that no intact cells were present in the pigment preparation. A squid melanin suspension was prepared by the cold HCl hydrolysis of whole squid ink followed by dialysis. Forty-eight hours after adding the pigment particles to the epidermal cell culture, the cover slip with the attached cells was stained with hematoxylin and eosin and examined under the oil immersion lens. The melanoma pigment particles appeared as ellipsoidal particles, just below the resolution limit of the optical microscope. Many of these particles were found to be located apparently inside epidermal cells (Fig. 1). Careful focusing gave convincing evidence that the pigment particles were not in the plane of the microscope slide, and hence not lying beneath the epidermal cells. The same technique suggested that the particles were not simply lying on the upper surface of the cells, and the failure to observe particles adhering to the sides of cells supported the conclusion that the particles were in fact lying entirely within the cytoplasm. To confirm this, a collection of cultured cells to which melanin particles were added 48 hours previously, were compacted gently in the centrifuge, the supernatant was decanted, and an osmic acid fixative was added. The pellet of cells was subsequently imbedded, sectioned, and observed in a Phillips electron microscope (Model EM-75), and the intracellular (and extranuclear) location of the pigment particles was confirmed. The same phagocytic behavior was observed when squid

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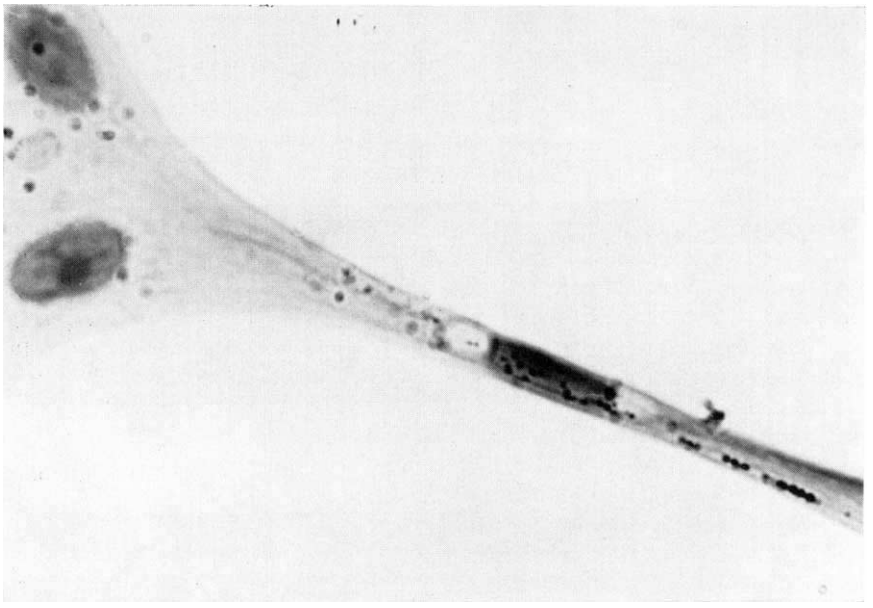

FIG. 1. Photomicrograph [oil immersion] showing portions of three human epidermal cells in culture. The melanin granules seen in the cytoplasm were obtained from a pigmented mouse melanoma and added to the culture 48 hours before fixation of the cells.

melanin particles were employed. Observations of control cultures showed the presence of occasional dendritic cells which may have been melanocytes carried into the culture but no pigment granules could be found in the control cultures, either intra- or extra-cellularly.

DISCUSSION

In those cell cultures to which the pigment particles were added, phagocytosis of the particles was the dominant process and only with difficulty could cells be found which did not contain the particles. Although the cultures presumably contained some melanocytes and mesenchymal cells as well, probably 90% or more of the population were epidermal cells, so that specific cell identification was not necessary in order to establish that these cells were indeed phagocytizing the particles. Since the biological sources of the melanin particles employed were from widely different species it could be inferred that this phagocytosis was non-specific, which in fact appears to be the case since carbon particles (PeliKan—india ink) were also observed to be phagocytized.

SUMMARY

In the *in vitro* cell culture of a population of primarily epidermal cells, it was observed that the melanin granules (more precisely, the fully-melanized melanosomes) obtained from a mouse melanoma and from squid ink, when added to the cell culture were actively phagocytized. It was also noted that india ink particles were phagocytized under similar conditions.

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